WADC TECHNICAL REPORT 54-30

THE INVESTIGATION OF FUNGICIDES FOR LEATHER

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MATERIALS LABORATORY

FEBRUARY 1954

WRIGHT AIR DEVELOPMENT CENTER

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Materials Laboratory

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FOREWORD

This report was prepared by the Materials Laboratory. The project was initiated under Research and Development Order No. 611-15 (A-H), "Preservative Chemicals", and was administered under the direction of the Directorate of Research, Wright Air Development Center with 1st/Lt Martin A. Townsend and Capt Paul A. Albert acting as project engineers.

ABSTRACT

Forty-two experimental formulations containing fungicidal chemicals have been evaluated as protective treatments against mildew on leather. Twenty-one were formulations containing orthophenylphenol, eleven formulations contained fluorinated compounds, three formulations contained trichlorophenyl acetate, three formulations contained paranitrophenol, two formulations contained parachlorometaxylenol, and the remaining two formulations contained di-lauryl dimethyl ammonium bromide and 2,2 dihydroxy -5,5' dichlorodiphenyl methane, as the active fungicides. To determine the fungistatic effectiveness of each treatment, at least one of two methods was employed; the Petri plate method (mycelial mat), and the mixed spore suspension method. With the Petri plate method, only the fungus Aspergillus niger was used. With the mixed spore suspension method fourteen fungi were used.

All of the treatments inhibited the growth of fungi to some degree, with the exception of the formulations containing parachlorometaxylenol, 2,2' dihydroxy -5,5' - dichlorodiphenyl methane, and di-lauryl dimethyl ammonium bromide as the active fungicides.

In addition, most of the treatments which inhibited the growth of fungi were no more corrosive than the untreated leather, when in contact with cadmium plated steel, clad 24S-T3 aluminum alloy and brass.

Toxicity evaluations of orthophenylphenol and the fluorinated benzene derivatives show that these fungicides are non-toxic under the conditions studied.

PUBLICATION REVIEW

This report has been reviewed and is approved.

FOR THE COMMANDER:

M. E. SORTE
Colonel, USAF
Chief, Materials Laboratory
Directorate of Research

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INTRODUCTION

This research project was undertaken to develop a fungistatic treatment for use on leather items involving intimate, prolonged skin contact.

The fungistatic treatment in use at the time by the Air Force, namely the active fungicide paranitrophenol, was considered unsatisfactory due to its toxicity when in contact with the skin.

Orthophenylphenol, properly formulated and applied, was found to be an effective fungistatic treatment, as well as non-toxic to the skin at fungistatic concentrations. Results also indicate that the fluorinated compounds, properly formulated and applied, are effective fungicides for leather.

A simple method of analysis for determining the presence of orthophenylphenol was developed by Caulfield 1/. A workable method of analysis for determining the presence of the fluorinated nitrobenzenes is being investigated.

The data obtained with forty-two fungistatic treatments that have been examined during the period from 15 August 1951 to 15 Jan. 1954 are reported herein.

1/ Calufield, P. H., and Robinson, R. J., Spectrophotometric determination of O-phenylphenol with titanium sulfate. Anal. Chem., Volume 25, 982 (1953)

EXPERIMENTAL PROCEDURES

Petri Plate Method (Mycelial Mat)

To prepare Petri plates for the mycelial mat tests a sufficient quantity of agar was made up, using the indicated amounts of each ingredient:

NH_4NO_3	3.00 grams
$K_2 HPO_4$	1.00 grams
$Mg SO_4 \cdot 7H_2O$	0.25 grams
KCl	0.25 grams
Sucrose or Glucose	20.00 grams
Agar	15.00 -20.00 grams
Distilled Water	100.00 grams

The ingredients were placed in a suitable flask (plugged with cotton), and the medium melted in an autoclave. The pH was adjusted to 5.5-6.5, with HCl or NaOH, and the plug replaced. The medium was then sterilized in an autoclave for 20 minutes at 121°C. Using aseptic techniques, 25 cc. of culture medium were poured into each 10-cm. sterile petri plate, and allowed to harden.

Preparation of Inoculum

To prepare the inoculum, five ml. of sterile, distilled water, containing 0.005 of one percent of the non-toxic wetting agent, dioctyl sodium sulfosuccinate, were added to a test tube of the organism Aspergillus niger USDA 215-4247, which was in an actively growing, spore producing condition. Using a transfer needle, the surface of the culture was scraped gently to bring spores into suspension. The suspension was then poured into about 35 ml. of sterile, distilled water, containing 0.005 percent of the same non-toxic wetting agent, and shaken to thoroughly disperse the spores.

Inoculation

The hardened culture medium was inoculated (using aseptic techniques) by first loading a sterile camel's hair brush with spores, from the spore suspension above, and then brushing the surfaces of the culture medium uniformly. After this, the prepared plates were incubated at a temperature of 30° ± 2° Centigrade and relative humidity of 95 ± 5 percent, until the white mycelium of the fungus was evident over the entire surface of the hardened culture medium.

Preparation of Leather Specimens for Agar Plate Test

Following the compounding of the treating solutions, those leather samples for testing the fungistatic properties of the formulations were processed by dipping the samples into the solutions for three minutes, then air dried. Three specimens of treated leather and one specimen of untreated leather, each two inches square, were shaken in twenty times their weight of distilled water for three hours. The same number of untreated and treated specimens of leather were moistened, only, by immersion for 5 - 10 minutes in distilled water. After the excess water was removed from all of the specimens, by blotting, one specimen was placed firmly on the mycelial mat in each dish, and the dishes were incubated at a temperature of 30 $\pm$ 2° Centigrade and relative humidity of 95 $\pm$ 5 percent for 21 days.

Mixed Spore-Sand Method

This method, which is considered easier to accomplish than, and superior to, the petri plate method, does not require the use of a culture medium, or aseptic techniques.

Description of Inoculum

The inoculum consists of a mixture of fungus spores and sand. Spores from fourteen different organisms are included. These fungi are Aspergillus niger, A. repens, A. flavus, A fumigatus, A. terreus, Paecilomyces varioti, Rhizopus arrhizus, Myrothecium verrucaria, Gliocladium fimbriatum, Penicillium pinophilum, P. luteum, P. oxalicum, P. spinulosium, and P. namylowskii. The inoculum is known as the American Leather Chemists Association, Spore Mixture, and was obtained from the Tanners' Council Laboratory, University of Cincinnati.

Preparation of Leather Specimens for Mixed Spore Inoculation Test

Two specimens of treated leather and one specimen of untreated leather, each one inch square, were shaken in twenty times their weight of distilled water for three hours (drummed). The same number of untreated and treated specimens of leather were moistened, only, by immersion for 5 - 10 minutes in distilled water. After the excess water had been removed by blotting, the moist specimens were inoculated by lightly dusting them with the fungus spore mixture. The treated and untreated specimens were suspended in separate containers, and all of the specimens were incubated for 30 days at 30 $\pm$ Centigrade and 95 $\pm$ 5% relative humidity.

Corrosion Tests

Corrosion tests were conducted on treated and untreated samples of finished vegetable tanned sheepskin using brass, clad aluminum 24S-T3 and cadmium plated steel. Orthophenylphenol and the fluorinated compounds were the fungicides used.

To conduct the corrosion tests, leather-metal sandwiches were made by placing the treated leather to the abraded surfaces of the metal and clamping with a pressure of 40 to 50 psi to hold each sandwich firm. The test sandwiches were stored at a temperature of 100°F. and 95 ± 2 percent relative humidity. Observations for evidence of corrosion were made at the end of 7 and 14 day periods.

Toxicity Evaluation

The skin toxicity effects on people of orthophenylphenol in leather were investigated by the United States Public Health Service, through the Office of the Air Surgeon, using samples of finished and unfinished vegetable-tanned sheepskin, and chrome-tanned horsehide.

Work on the toxicological evaluation of the four fluorinated benzene derivatives used in this study was accomplished by skin patch testing of guinea pigs and 351 human volunteers using fabric impregnated with the fluorine compounds in concentrations as follows:

1-fluoro-3-bromo-4,6-dinitrobenzene -	0.6% by weight
1-fluoro-3-chloro-4,6-dinitrobenzene -	0.2% by weight
1,3-difluoro-4,6-dinitrobenzene -	0.4% by weight
1-fluoro-2,4-dinitrobenzene -	0.2% by weight

Acetone solvent treated control.

These investigations were conducted by the University of Michigan, Institute of Industrial Health on Air Force Contract 33(616)-2049, through the Toxicology Section of the Aero Medical Laboratory at this Center.

DISCUSSION OF RESULTS

Results obtained with both the Petri plate method and the mixed spore suspension method were determined by observations at frequent intervals. These observations were recorded as none, slight, moderate or heavy fungus growth, in tabular form.

Orthophenylphenol

The fungistatic effect of orthophenylphenol was investigated in several types of leather, as shown in Table 1. Leather samples with fungicide contents ranging from 0.28% to 3.46% were evaluated. Comparable results were obtained by both the Petri plate and mixed spore suspension methods. Orthophenylphenol was most effective in finished vegetable-tanned sheepskin and finished chrome-tanned horsehide, showing 100% inhibition at deposits of 1.38% and 1.4% respectively, whereas unfinished vegetable-tanned sheepskin at a deposit of 1.30% supported considerable growth after

14 days of incubation. However, when the concentration of orthophenylphenol in unfinished vegetable-tanned sheepskin was increased to 3.30%, 100% inhibition was obtained via both methods of evaluation.

Vegetable-tanned sheepskin was used in the investigations for two reasons, namely, (1) it is the type of tanned leather used in hat sweat bands; (2) it is highly susceptible to fungus growth.

Essentially two basic formulations of orthophenylphenol were investigated. These were (1) orthophenylphenol, Stoddard's solvent, and sulfur chlorinated rape seed or soybean oil; (2) orthophenylphenol, Stoddard's solvent, and silicone resin. As indicated in Appendix I, Table 1, the first formulation was more effective.

Fluorine Compounds

Four fluorinated compounds were investigated, as shown in Appendix I, Table 2. Each of these were formulated with Stoddard's solvent and sulfur-chlorinated vegetable oil, with the exception of 1-fluoro-3-bromo 4,6 dinitro-benzene, which was formulated with Stoddard's solvent and silicone resin. All of them exhibited 100% inhibition to the growth of the fungi used in the mixed spore suspension method, at very low concentration. The concentrations ranged from 0.05% (1-fluoro-3-bromo 4,6 dinitrobenzene) to 0.19% (1-3 difluoro 4-6 dinitro benzene). Since lower concentrations were not evaluated, they may be even more effective.

The deposit of 2-4 dinitro fluoro benzene was not known. The low concentration of the compound in the treating solution (0.5%) did indicate that its effectiveness was very high.

Trichlorophenyl Acetate

To compare the fungistatic activity of trichlorophenyl acetate with orthophenylphenol, leather samples were treated with solutions containing 1.5,2.0 and 5.0 percent concentrations of trichlorophenyl acetate in the Stoddard solvent-sulfur chlorinated vegetable oil formulations.

The petri plate method was used in the evaluation of all three concentrations, and the mixed spore suspension method was used only in the evaluation of the 5.0% concentration.

As shown in Appendix I, Table 3, a concentration of 5.0% in the treating solution was highly effective, giving results comparable to those obtained in the investigation of orthophenylphenol.

Miscellaneous Compounds

Several compounds in various formulations were investigated, with only paranitrophenol exhibiting 100% inhibition to the growth of fungi, as shown in Appendix I, Table 4, using the mixed spore suspension method. A formulation containing the compound 2,2' dihydroxy-5,5' dichloro diphenyl methane actually supported the growth of fungi sooner than the same leather, untreated.

Paranitrophenol, using a Stoddard's solvent-silicone resin formulation was shown to be much more effective than orthophenylphenol, at the same concentration, and in the same formulation. The toxicity to people of paranitrophenol prevents its use where intimate skin contact is involved.

Di-lauryl dimethyl ammonium bromide and parachlorometaxylenol exhibited practically no fungistatic effects, although only one concentration of each was evaluated.

Corrosion Effects

The results of the corrosion tests on orthophenylphenol and the fluorinated benzene derivatives are recorded in Appendix I, Tables 5 and 6. The results show that leather specimens treated with the above compounds have no more corrosive properties than the corresponding untreated leather control specimen - and, with the clad aluminum 24S-T3, the treated leather specimens produced less pitting than the untreated control.

Toxicity Effects

The results of the toxicity evaluation of orthophenylphenol in leathers are recorded in Appendix I, Table 7. Apparently, the "not acceptable" results obtained with the finished, treated and untreated vegetable-tanned sheepskin, and the untreated, finished, chrome-tanned horsehide are due to some material used in the finishing process having a toxic dermatological effect. The only explanation that can be proposed for the "acceptable" result obtained with the finished, chrome-tanned horsehide treated to contain 1.40% orthophenylphenol is that the sulphur-chlorinated vegetable oil masked the known toxic effects of the chrome leather.

A preliminary report on the patch testing results obtained for toxicological evaluation of the four fluorinated compounds, used in previously described leather treatment formulations, indicated that these chemicals are not primary irritants or sensitizers. Twenty-five human volunteers and six guinea pigs were used in the above preliminary tests. A later report, using 326 human volunteers, confirmed the above findings.

CONCLUSIONS

It has been determined that leather treated with the fungicide orthophenylphenol formulated with sulphur chlorinated vegetable oil and a hydrocarbon solvent so that the leather contains approximately 1.6 percent by weight fungicide is thoroughly protected against mold producing fungi. Leather treated with this fungicide to contain the amount indicated has been shown to be satisfactory for intimate prolonged skin contact for personnel. The treated leather was shown to be no more corrosive to clad 24S-T3 aluminum alloy, cadmium plated steel or brass, than the untreated leather.

Fluorine compounds formulated in the sulphur chlorinated vegetable oil were fungicidal at much lower concentrations than the orthophenylphenol and were no more corrosive to the above named metals than the untreated leather. Further investigations will be made by the Biochemical Section, Materials Laboratory, WADC, to develop an applicable formulation for use in a leather preservative, as the need arises.

Trichlorophenyl acetate proved to be fungicidal in leather when formulated in vegetable oil at a concentration of 5 percent by weight. No additional investigations will be made at this Center on trichlorophenyl acetate because of the high concentration required to prevent the growth of fungi.

A method of analysis was developed for determining orthophenylphenol in leather.

Results of patch testing obtained with the four fluorinated benzene derivatives indicate that the compounds are neither skin irritants nor sensitizers.

APPENDIX I

THE DETERMINATION OF ORTHOPHENYLPHENOL IN LEATHER

Materials

Anhydrous Diethyl Ether, CP

Titanium Syrup.- 450 milligrams of anhydrous CP titanium dioxide is dissolved in one liter of concentrated sulfuric acid, specific gravity 1.84. (This requires heating for eight hours.) The resulting solution should be clear and have a small but constant optical density through the 350-800 millimicron region.

Stock Solution

Orthophenylphenol, 100 milligrams in 100 milliliters of diethyl ether.

Equipment

1.- Colorimeter - The Cary Model 11 spectrophotometer, The Beckman DU spectrophotometer or equal type are satisfactory.
1 - 25 ml. burette and stand.
4 - 125 ml. flasks.
4 - 250 ml. beakers.
1 - 100 ml. volumetric flask.
4 - normal funnels and stand.

Procedure.- Select 2 random samples of treated leather and 2 samples of untreated leather. Each original sample shall weigh approximately 5 grams. Keeping samples separate, cut each sample into small pieces approximately 1 mm. square and dry for 16 hours at 40° C.

From each sample take a random portion of such weight so that the calculated amount of orthophenylphenol will be between 1 and 3 milligrams in each sample to be analyzed. (For example if the leather contains from 1.0 to 3.5 percent orthophenylphenol a 0.100 gram sample is recommended.)

Each sample is placed in a separate, properly labeled, 125 ml. flask. To each flask add 25 ml. of anhydrous ether. The flasks are stoppered and allowed to stand overnight or about 16 hours. The ether extract is then decanted through suitable filter paper into 250 ml. beakers. A second extraction is made by adding 15 ml. ether to each sample and shaking occasionally for 15 minutes and decanting the liquid to the first extraction. A third and similar extraction will insure that all of the fungicide is removed from the leather. Evaporate the ether with or without the aid of vacuum but no heat should be used. The evaporation should continue if vacuum is used until about 10 ml. of concentrated extract remains. These last few milliliters must be evaporated under room conditions about 20° C. without the use of vacuum to avoid loss of the orthophenylphenol that remains in the residue.

Add 25 ml. of the titanium syrup at 20° C. to the fungicide residue and stir the resulting solution for 5 minutes. The titanium syrup must be added immediately after the ether is evaporated and water must be absent since it reduces the optical density of the resulting titanium-phenol complex. After about 15 minutes the titanium-phenol mixture in a suitable cell is examined with the spectrophotometer at 455 millimicrons. (The titanium-phenol complex is stable up to 2 hours.)

The average optical density of the extract from the untreated leather is subtracted from the average optical density of the extract from the treated leather and this is used to determine the concentration of orthophenylphenol in the leather from a standard curve.

A standard curve for 0-4 milligrams orthophenylphenol is desirable. Using stock solution described above, aliquots are taken for the desired amounts of phenol. The ether solvent is evaporated and 25 ml. of the titanium syrup added to the residue as described above.

The optical density values at 455 millimicrons are plotted on standard cross section paper as a function of the concentration of orthophenylphenol.

Frequent checking of the standardization curve is recommended.

Appendix I,
Table 1 - Fungistatic Effect of Orthophenylphenol in Leather

Deposit	Solvent	Type of Leather	Conditioning Process	Aspergillus niger (Petri Plate Method)			A.L.C.A. Sand Spore Mixture (Mixed Spore Suspension Method)				
				7 days	14 days	21 days	7 days	14 days	21 days	28 days	30 days
0.65%	(Stoddard's Solvent and) (S.Cl. Vegetable Oil)	Finished, Vegetable-Tanned Sheepskin	Drummed	1	--	--	2	3	3	3	3
"	"	"	Not Drummed	1	--	--	2	3	3	3	3
1.38%	"	"	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0
1.51	"	"	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0
1.77%	"	"	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0
3.75%	"	"	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0
Not Treated	---	"	Drummed	3	3	3	1	3	3	3	3
"	---	"	Not Drummed	3	3	3	1	3	3	3	3
1.30%	(Stoddard's Solvent and) (S.Cl. Vegetable Oil)	Unfinished, Vegetable-Tanned Sheepskin	Drummed	1	3	3	1	3	3	3	3
"	"	"	Not Drummed	1	3	3	3	3	3	3	3
3.30%	"	"	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0
3.45%	"	"	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0
3.46	"	"	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0
Not Treated	"	"	Drummed	3	3	3	3	3	3	3	3
"	"	"	Not Drummed	3	3	3	3	3	3	3	3
2.50%	(Stoddard's Solvent and) (S.Cl. Vegetable Oil)	Finished Bark-Tanned Strap Leather	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0
2.64	"	"	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0
Not Treated	"	"	Drummed	3	3	3	2	3	3	3	3
"	"	"	Not Drummed	3	3	3	0	0	3	3	3

Key 0 - No growth
1 - Slight growth
2 - Moderate growth
3 - Heavy growth

Table 1 (Continued)

Deposit	Solvent	Type of Leather	Conditioning Process	Aspergillus niger (Petri plate Method)			A.L.C.A. Sand Spore Mixture (Mixed Spore Suspension Method)				
				7 days	14 days	21 days	7 days	14 days	21 days	28 days	30 days
1.4%	(Stoddard's Solvent (and S.Cl. Vegetable Oil)	Finished Chrome-Tanned Horsehide	Drummed	0	0	---	---	---	---	---	---
"	"	"	Not Drummed	0	0	---	---	---	---	---	---
3.4%	(Stoddard's Solvent) (and Silicone Resin)	"	Drummed	---	---	---	0	0	0	0	0
"	"	"	Not Drummed	---	---	---	0	0	0	0	0
Not Treated	"	"	Drummed	---	---	---	0	1	1	3	3
"	"	"	Not Drummed	---	---	---	0	1	1	3	3
0.28%	(Stoddard's Solvent and) (S.Cl. Vegetable Oil)	Finished Vegetable-Tanned Cowhide	Drummed	---	---	---	1	3	3	3	3
"	"	"	Not Drummed	---	---	---	3	3	3	3	3
Concentration in Treating Solution											
0.75%	(Stoddard's Solvent and) (Silicone Resin)	Finished Vegetable-Tanned Sheepskin	Drummed	---	---	---	0	2	2	3	3
"	"	"	Not Drummed	---	---	---	0	2	2	3	3
5.00%	"	"	Drummed	---	---	---	0	1	1	3	3
"	"	"	Not Drummed	---	---	---	0	0	0	0	0
5.00%	(Stoddard's Solvent and)										
"	(S.Cl. Vegetable Oil)	"	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0
0.75%	(Stoddard's Solvent and) (Silicone Resin)	Finished Bark-Tanned Strap Leather	Drummed	---	---	---	0	2	2	3	3
"	"	"	Not Drummed	---	---	---	0	2	2	3	3
2.00	Shoe Polish	"	Drummed	3	3	3	1	1	3	3	3
"	"	"	Not Drummed	3	3	3	1	1	3	3	3
5.00%	Stoddard's Solvent/ Silicone Resin	"	Drummed	---	---	---	0	0	0	0	0
"	"	"	Not Drummed	---	---	---	0	0	0	0	0

Key 0 - No growth
1 - Slight growth
2 - Moderate growth
3 - Heavy growth

Appendix I,
Table 2 - The Fungistatic Effect of Fluorinated Compounds in Leather

Deposit	Compound	Solvent	Type of Leather	Conditioning Process	A.L.C.A. Sand Spore Misture (Mixed Spore Suspension Method) 7 days	14 days	21 days	28 days	30 days
0.05%	1 - Fluoro - 3 - Bromo- 4,6 Dinitro Benzene	(Stoddard's Solvent) (and Silicone Resin)	Finished Vegetable-Tanned Sheepskin	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0
0.14%	"	"	Finished Chrome-Tanned Horsehide	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0
0.14%	(1 - Fluoro - 3 - Chloro-) (4,6 Dinitro Benzene)	(Stoddard's Solvent) (and S.Cl. Vegetable Oil)	Finished Vegetable-Tanned Sheepskin	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0
0.19%	(1,3 Difluoro - 4,6-) (Dinitro Benzene)	"	"	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0
Concentration in Treating Solution									
	(1 - Fluoro - 3 - Bromo-)	(Stoddard's Solvent)							
0.50%	(4,6-Dinitro Benzene)	(and Silicone Resin)	"	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0
"	"	"	Finished Chrome-Tanned Horsehide	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0
	(1 - Fluoro - 3- Chloro-)	(Stoddard's Solvent)	Finished Vegetable-						
"	(4,6 Dinitro Benzene)	(and S.Cl. Vegetable Oil)	Tanned Sheepskin	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0
"	"	"	Finished Vegetable-Chrome-Tan. Cowhide	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0
"	(1,3 - Difluoro - 4 - 6) (-Dinitro Benzene)	"	Finished Vegetable-Tanned Sheepskin	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0

Key 0 - No growth
1 - Slight growth
2 - Moderate growth
3 - Heavy growth

Table 2 (Continued)

Deposit	Compound	Solvent	Type of Leather	Conditioning Process	A.L.C.A. Sand Spore Mixture (Mixed Spore Suspension Method) 7 days	14 days	21 days	28 days	30 days
0.50%	(1,3 - Difluoro -4-6-) (Dinitro Benzene)	(Stoddard's Solvent) (and S.Cl. Vegetable Oil)	Finished Chrome-Tanned Cowhide	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0
"	(1 - Fluoro-2,4-Dinitro) (Benzene)	"	Finished Vegetable-Tanned Sheepskin	Drummed	0	0	0	0	0
"	"	"	"	Not Drummed	0	0	0	0	0
Untreated	_____		"	Drummed	3	3	3	3	3
"	_____		"	Not Drummed	3	3	3	3	3
"	_____		Finished Chrome-Tanned Horsehide	Drummed	0	1	1	3	3
"	_____		"	Not Drummed	0	1	1	3	3
"	_____		Finished Chrome-Tanned Cowhide	Drummed	1	2	3	3	3
"	_____		"	Not Drummed	1	2	3	3	3

Key
0 - No growth
1 - Slight growth
2 - Moderate growth
3 - Heavy growth

Appendix I, Table 3 - The Fungistatic Effect of Trichlorophenyl Acetate in Leather

Concentration in Treating Solution	Solvent	Type of Leather	Conditioning Process	Aspergillus niger (Petri Plate method)			A.L.C.A. Sand Spore Mixture (Mixed Spore Suspension Method)				
				7 days	14 days	21 days	7 days	14 days	21 days	28 days	30 days
1.5%	(Stoddard's Solvent and) (S.Gl. Vegetable Oil)	Finished Vegetable-Tanned Sheepskin	Drummed	1	3	3	---	---	---	---	---
"	"	"	Not Drummed	1	3	3	---	---	---	---	---
2.0%	"	"	Drummed	1	1	1	---	---	---	---	---
"	"	"	Not Drummed	1	1	1	---	---	---	---	---
5.0%	"	"	Drummed	0	0	0	0	0	0	0	0
"	"	"	Not Drummed	0	0	0	0	0	0	0	0

Key 0 - No growth
1 - Slight growth
2 - Moderate growth
3 - Heavy growth

Appendix I, Table 4 - Fungistatic Effect of Miscellaneous Compounds in Leather

Concentration in Treating Solution	Compound	Solvent	Type of Leather	Conditioning Process	A.L.C.A. Sand Soore Mixture 7 days	14 days	21 days	28 days	30 days
2.0%	(Di-lauryl dimethyl) (ammonium bromide)	(Stoddard's Solvent and) (S.O1. Vegetable Oil)	Finishes Vegetable-Tanned Sheepskin	Drummed	2	3	3	3	3
"	"	"	"	Not Drummed	2	3	3	3	3
0.5%	(2,2' dihydroxy-5,5') (dichlorodiphenyl) (Methane)	Emulsion	"	Drummed	3	3	3	3	3
"	"	"	"	Not Drummed	3	3	3	3	3
0.125%	Paranitrophenol	Stoddard's Solvent/ Silicone Resin	"	Drummed	0	0	0	0	0
				Not Drummed	0	0	0	0	0
"	"	"	Finished Bark-Tanned Strap Leather	Drummed	0	0	0	0	0
				Not Drummed	0	0	0	0	0
0.125%	Parachlorometaxylenol	"	"	Drummed	1	3	3	3	3
"	"	"	"	Not Drummed	3	3	3	3	3
"	"	"	Finished Vegetable-Tanned Sheepskin	Drummed	2	3	3	3	3
				Not Drummed	3	3	3	3	3
Deposit									
0.3%	Paranitrophenol	_____	Finished Bark-Tanned Strap Leather	Drummed	0	0	0	0	0
"	"	_____	"	Not Drummed	0	0	0	0	0
"	Untreated	_____	Finished Vegetable-Tanned Sheepskin	Drummed	3	3	3	3	3
"	"	_____	"	Not Drummed	3	3	3	3	3

Key
0 - No growth
1 - Slight growth
2 - Moderate growth
3 - Heavy growth

APPENDIX I, Table 5

Results of Corrosion Tests Conducted on Samples of Finished Vegetable-Tanned Sheepskin at a Temperature of 100° F. and 95 ± 5% R.H.

Percent Deposit	Treatment Compound	Corrosion Test Results		
		Clad Aluminum 24S-T3	Brass	Cadmium Plated Steel
--	Untreated	Severe Pitting	Stain - No Pitting	No pitting
1.38	Orthophenyl-phenol	Less pitting than untreated	"	"
1.51	"	"	"	"
1.77	"	"	"	"

APPENDIX I, Table 6

Results of Corrosion Tests Conducted on Samples of Finished Vegetable-Tanned Sheepskin at a Temperature of 100° F. and 95 ± 2% R.H.

Percent Deposit	Treatment Compound	Corrosion Test Results		
		Clad Aluminum 24S-T3	Brass	Cadmium Plated Steel
--	Untreated	Severe Pitting	Dezincification	No Significant Pitting
0.05	1-Fluoro-3-bromo-4,6-dinitrobenzene	Less Pitting Than Untreated	"	"
0.14	1-Fluoro-3-chloro-4,6-dinitrobenzene	"	"	"
0.19	1,3-Difluoro-4,6-dinitro-benzene	"	"	"

APPENDIX I, Table 7

The Toxicity Evaluations of Orthophenylphenol in Leather - For Use in Items Involving Intimate, Prolonged Skin Contact.

Deposit	Type of Leather	Result
Untreated	Unfinished Vegetable-Tanned Sheepskin	Acceptable
1.61%	" " " "	Acceptable
Untreated	Finished Vegetable-Tanned Sheepskin	Not Acceptable
1.175%	" " " "	Not Acceptable
Untreated	Finished Chrome-Tanned Horsehide	Not Acceptable
1.40%	" " " "	Acceptable